Amino acid chronology of the Lower Pleistocene deposits of Venta Micena (Orce, Granada, Andalusia, Spain)

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Abstract—This paper deals with a model adjustment for the estimation of age by means of amino acid racemization analysis. Two model families were obtained on the basis of the different genera of molluscs analysed, and were applied to a palaeontological site located in the Cúllar-Baza basin: "Venta Micena" (Orce, Granada). The analytical results obtained from the study of fossil gastropods have provided a very coherent average dating of 983 ± 58 Ky, this coinciding to a large extent with the most widespread palaeontological, geological and stratigraphical datings for the site. There is, however, no agreement with certain recent theories that situate Venta Micena chronologically at the Pliocene–Pleistocene boundary (ca. 1.6 My) or before. The validity of the dating fit was tested on the basis of another classical palaeontological site, Cúllar-Baza, where a coherent dating of 441 ± 27 Ky was obtained.

INTRODUCTION

One of the most important problems involved in Pleistocene palaeoenvironmental and palaeoclimatological studies in Spain, and other parts of the world, is that of placing the observations on a chronostratigraphical scale allowing the velocity or isochrony of the processes to be established. Traditionally, relative dating has been accomplished through geomorphological, palaeontological and archaeological studies in which forms, fossils and artifacts were placed on the stratigraphical scale according to (more or less) subjective analysis, i.e. the relative topographical level of fluvial terraces or glacial moraines, the evolutionary stage of fossil remains, according to an assumed velocity of evolution or the morphological characteristics of stone artifacts. Obviously these methods do not work accurately because in most cases there is in fact a feedback process: forms and deposits are used to place fossil and archaeological remains on the chronostratigraphical scale and later provide the clue for dating of the processes.

Radiometric *s.l.* methods (^{14}C, U-series, K/Ar, ESR, TL...) are particularly useful tools for dating and are widely used in spite of the range method limitations; however, the most frequent problem is related to the possibility of finding samples for dating purposes (weight or nature). The amino acid racemization dating method provides certain advantages: only minor sampling processes are required (80 mg maximum) and a wide group of materials are available for dating, e.g. shells from mollusca (Goodfriend, 1987; Wehmiller, 1993), foraminifera (Wehmiller, 1993), ostracoda (Torres *et al.*, 1995) and eggs (Meyer, 1992); bones and teeth (Bada *et al.*, 1973a; Marzin, 1990; Elster *et al.*, 1991), wood (Rutter and Vlahos, 1988), speleothemes (Lauritzen *et al.*, 1994), or even rocks and soft sediments (Hearty *et al.*, 1992).

The amino acid analysis of fossils was initiated many years ago (Abelson, 1954) and has since been continued by many researchers (Hare, 1969, 1971; Bada and Protchs, 1973; Bada and Schroeder, 1972; Miller and Hare, 1975). Today, amino acid racemization ratio analysis makes it possible to determine the palaeoclimatological data of a region (Bada *et al.*, 1973b; Wehmiller, 1993). Amino acid racemization analysis also allows aminostratigraphy (Miller and Hare, 1980) and aminochronology to be established. Aminostratigraphy consists of arranging geological sites into a sequence on the basis of observed clusters of racemization ratios, established using representatives of one zoological genus. This method is an excellent tool for the correlation of sea level oscillation-linked deposits—marine and fluvial terraces indicative of warm and stable climate periods (Miller and Mangerud, 1985; Kaufman, 1992; Hearty *et al.*, 1992)—and is especially useful for the determination of neotectonics affecting Pleistocene deposits (Dumas *et al.*, 1988). In some cases, and mainly where almost continuous sedimentation has taken place, as in the Cúllar-Baza Basin, it is necessary to produce numerical-age results, the most common being age-calibrated

(^{14}C or U-series) results (Goodfriend, 1987; Dumas *et al.*, 1988; Hearty *et al.*, 1992; Wehmiller, 1993).

Some years ago, Venta Micena, a small-holding located close to Orce in Granada, was widely mentioned in the mass media as a result of a controversial finding of human/non-human remains (Gibert *et al.*, 1989, 1992b). It is not our objective here to get involved in palaeoanthropological questions, but rather to place this palaeontological site on a Pleistocene time-scale using the amino acid racemization dating method. This site, along with many others in the Cúllar-Baza Basin, was dated using this method during a lengthy dating campaign carried out for drawing up of the "Instituto Tecnológico Geominero de España" 1:50000 geological map (Torres, 1995).

EXPERIMENTAL

Geographical situation and palaeontological–geological settings

Venta Micena is a well-known paleontological site of Upper Villafranchian age (Fig. 1) located in the Guadix-Baza Basin. This basin consists of a long, narrow tectonically controlled depression, the major axis of which is oriented in a NE–SW direction. The Guadix-Baza Basin constitutes an exception to the behaviour of Iberian Peninsula Pleistocene basins: since at the end of Pliocene times they were all working under an incision-dominated regime, and in spite of short-term alluvial platform building, the erosion controlled by falling sea level was the origin of incomplete stratigraphical sections with alluvial or lacustrine terraced deposits, representing only warm (stable) intervals. During the Pliocene and most of the Pleistocene, the Guadix-Baza Basin operated as a classic endorheic basin, and there was stacking of a thick, almost continuous alluvial–lacustrine–palustrine sequence. The continuity of the Guadix-Baza Basin's Pliocene–Pleistocene geological record, and the large number of palaeontological localities preserved constitute a remarkable exception. Some authors (Anadón *et al.*, 1987; Agustí, 1984; Alberdi *et al.*, 1989) have established numerous chronostratigraphical sequences based on palaeontological data but without numerical dating.

Pliocene–Pleistocene sediments appear at the SW limit of the basin and at its northern boundary (ITGE, 1989). Lower Pleistocene deposits are well represented in the central part, whereas mid-Pleistocene materials are to be found at its southern limit. The Orce site has been wrongly placed in a Middle Pleistocene dated outcrop.

The Pleistocene sedimentation of the Orce (Venta Micena) area (Fig. 2) consists of fine-grained detri-

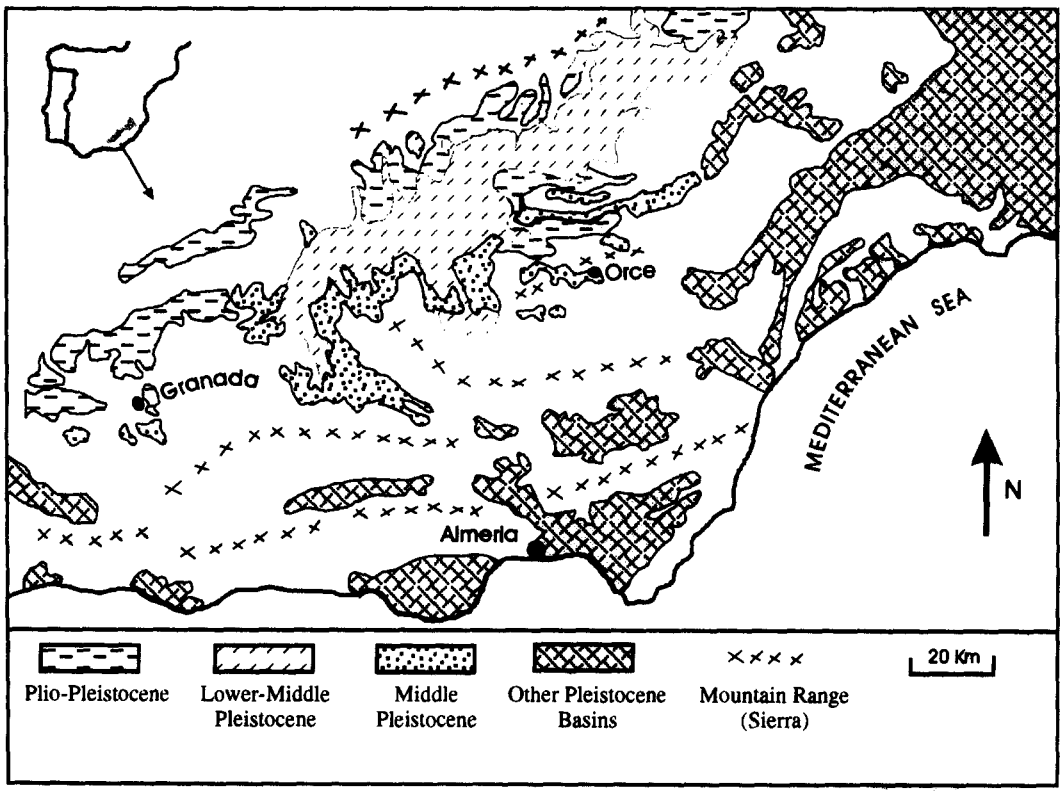

Fig. 1. Geographical and geological situation of Orce (Venta Micena) area (ITGE, 1989, modified).

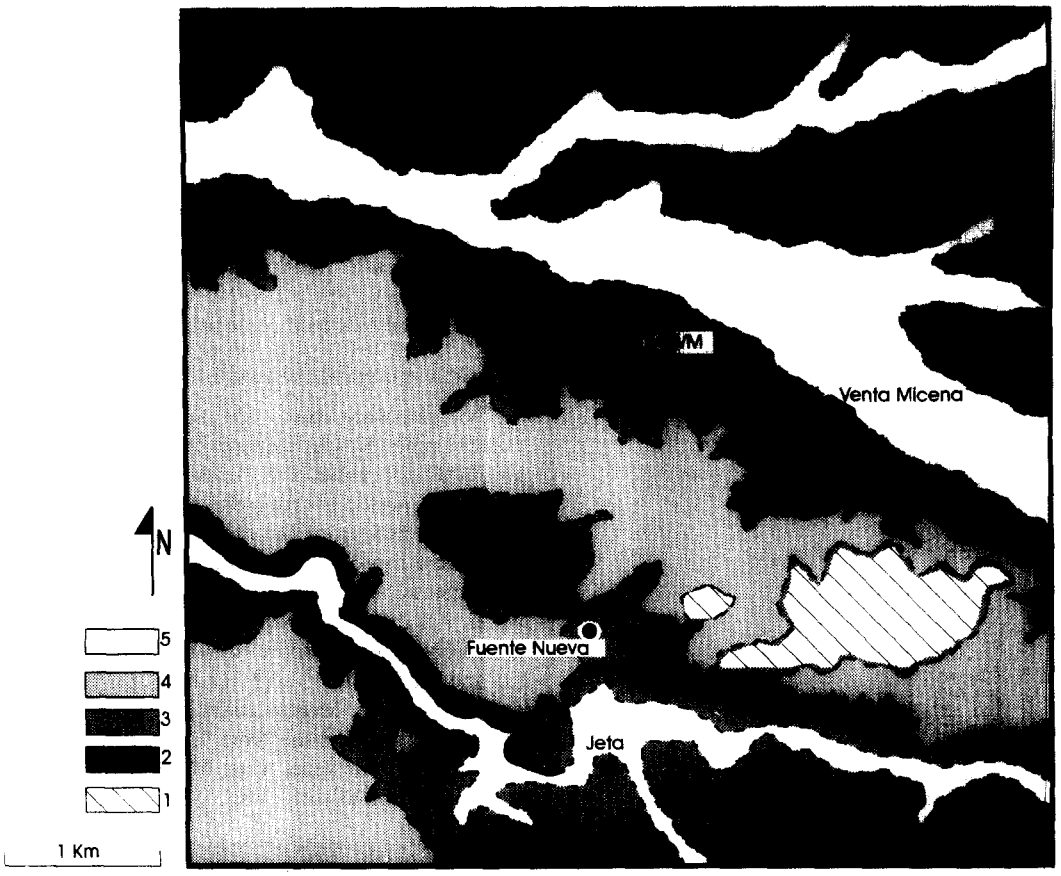

Fig. 2. Geology of the Venta Micena (VM) site (Soria *et al.*, 1987, modified). (1) Mesozoic rock threshold; (2) fluvial–alluvial fan Pleistocene deposits; (3) palustrine–lacustrine carbonate deposits with Venta Micena (VM) palaeontological site; (4) Middle and Upper Pleistocene alluvial deposits; (5) Holocene deposits.

tal deposits at the lower part (Soria *et al.*, 1987), followed by carbonate deposits of lacustrine origin. The lower-middle Pleistocene sediments were unconformably capped with recent gravels of alluvial origin. Mesozoic limestone outcrops acted as local thresholds during Pleistocene deposition. The bottoms of the valleys are filled with recent (Holocene) deposits.

The Venta Micena section (Anadón *et al.*, 1987) (Fig. 3) may be described as follows:

(a) dolomitic marls, dolostones and channelled gravel and sand;

(b) lutites and marls with abundant organic debris containing foraminifera, gastropoda and ostracoda, that have been sampled for amino acid racemization dating;

(c) slightly sandy lutitic limestones—the Venta Micena palaeontological site appears at the top of this stratum;

(d) partially covered, lutitic limestones and marls, dolostones and dolomitic marls—channelled gravel and sand are common;

(e) nodulous lutitic limestones and limestones with gastropoda casts.

The genesis of the Venta Micena palaeontological site (c) may be interpreted as a marginal deposit of the Cúllar-Baza Basin which occurred during a lacustrine expansion event overlapping a previous mud flat [(a) and (b)]; this lacustrine environment was temporarily interrupted by channelled deposits (d).

The Venta Micena vertebrate association is made up of the following: Ursidae (*Ursus etruscus*), Canidae (*Canis etruscus mosbachensis*, *Cuon priscus*, *Vulpes praeglacialis*, *Xenocyon* sp.), Felidae (*Homotherium latidens*, *Megantereon cultridens adroveri*, *Lynx* sp., *Panthera* cf. *gombaszogensis*), Hienidae (*Pachycrocuta brevirostris ruizi*), Perissodactyla (*Equus stenonis*, *Dicerorhinus etruscus*, *Hippopotamus incognitus*), Proboscidea (*Archidiskodon meridionalis*) and Artiodactyla (*Megaceros solhiacus*, *Cervus* (?) *elaphoides*, *Preaeovivos* sp., *Capra alba*, *Soergelia minor*, *Bison* sp.), as well as the controversial *Homo* sp. remains. There are also rodent and lagomorph remains

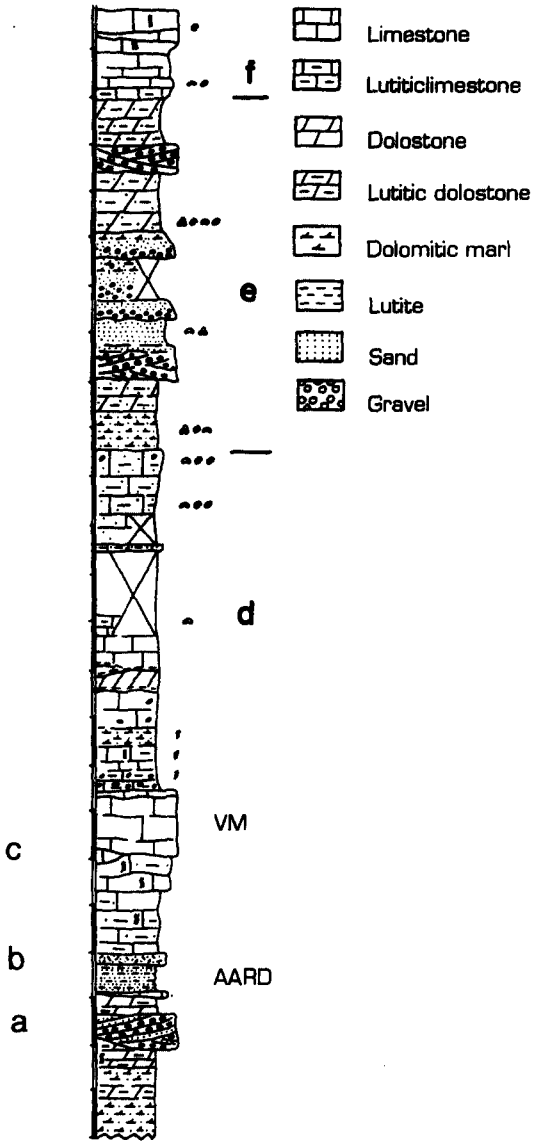

Fig. 3. Stratigraphical section of Venta Micena area (Anadón *et al.*, 1987, modified). VM fossil mammals bearing strata. AARD sampled bed for amino acid racemization dating.

(Agustí *et al.* (1987)): *Allophaiomys pliocaenicus*, *Castillomys crusafonti*, *Apodemus* aff. *mystacinus*, *Eliomis intermedius*, *Hystrix major* and *Prolagus* cf. *calpensis*; and many mollusca genera: *Melanoides*, *Melanopsis*, *Pomatias*, *Hydrobia*, *Mercuria*, *Hauffenia*, *Bithynia*, *Planorbis*, *Anisus*, *Gyraulus*, *Armiger*, *Ancylus*, *Acroloxus*, *Lymnaea*, *Cochlicopa*, *Vertigo*, *Pupilla*, *Helicella* and *Pisidium*, *cf.* Robles *et al.* (1987). Foraminifera and crustacea are also frequent.

According to palaeontological assemblages, and to the geomorphological characteristics and palaeomagnetism of the area, Venta Micena (Alberdi *et al.*, 1989) was dated near 1.3 My. Later (Gibert *et al.*, 1992a) an age of 1.6 My was established.

Sampling

A 5-kg sample was taken from a lutitic stratum located immediately below the Venta Micena fossiliferous horizon (Fig. 3). Gastropoda extraction was accomplished in accordance with the established process (Torres *et al.*, 1994). Twenty-three individual samples were picked out from the sieved sample and analysed: they proved to be six *Gastropoda indet. opercula* (Bithynia sp.), three *Radix (Lymnaea)* sp., one *Planorbis* sp., one *Bithynia* sp. and 12 *Fragmenta indet.* (probably *Bithynia* sp.)

Sample preparation and analysis

The glassware used for analysis (except the Pasteur pipettes) was cleaned by baking in an oven at 500°C for about 2 h. Eppendorf plastic micro test tubes, plastic micropipette tips and Pasteur pipettes were new from the factory. Teflon liners and septa were thoroughly washed with petroleum ether and acetone and rinsed three times with ultraclean water. All the water used in the analysis was Milli-Q quality from Millipore. Concentrated hydrofluoric and hydrochloric acids and trifluoroacetic acid anhydride were Merck analytical grade. Thionyl chloride was purchased from Fluka AG. Isopropyl alcohol and *n*-hexane were Merck HPLC grade, and dichloromethane was Merck spectroscopy grade.

All the shells used for amino acid racemization analysis were thoroughly cleaned by a combination of ultrasonication and mechanical cleaning with a dental drill, and rinsed thoroughly with deionized water, 2 N hydrochloric acid and again with deionized water. Hydrolysis of ca. 80 mg of shell was carried out in a mixture of 12 N hydrochloric acid (2.9 μl mg^{-1}) and 6 N hydrochloric acid (100 μl), in test tubes with Teflon-lined screw caps closed in an atmosphere of nitrogen, in a heating block at 100°C for 20 h. Desalting was accomplished in conical 1.5 ml Eppendorf plastic micro test tubes with caps, with concentrated hydrofluoric acid added (1.25 μl mg^{-1} of sample), this being mixed with a mechanical Vortex shaker, and centrifuged for 4 min in an Eppendorf centrifuge. The supernatant was transferred to new 1.5 ml Eppendorf micro test tubes, frozen in liquid nitrogen, and vacuum dried in a plastic desiccator. Samples were redissolved with 80 μl distilled water, mixed in the Vortex shaker, centrifuged for a few seconds to ensure the deposition of all the droplets, and then transferred to 2 ml glass vials with screw caps and Teflon-lined septa. Water was evaporated at vacuum from the vials in the plastic desiccator, the caps not being tightly closed.

The first amino acid derivatization step consisted of essterification with 250 μl of 3 M thionyl chloride in isopropanol. The vials were tightly closed in a nitrogen atmosphere and left to react on the heating block at 100°C for just 1 h. The tops of the vials

were then unscrewed (but not removed) in a hood and vacuum dried in a plastic desiccator just to the point of dryness. The second derivatization step consisted of *N*-trifluoroacetylation with 150 μl of trifluoroacetic acid anhydride (25% in dichloromethane). The vials were tightly closed in a nitrogen atmosphere and heated at 100°C for just 5 min on the heating block. They were subsequently allowed to cool and opened in a hood, where the dichloromethane solvent and the unreacted trifluoroacetic acid anhydride were evaporated under a gentle flow of nitrogen. Later the samples were dissolved in 125 μl of *n*-hexane, shaken in the vortex, and most of the *n*-hexane evaporated in a stream of nitrogen to a final volume of 15–25 μl, then being transferred to 150 μl injection vials.

One μl of sample was injected into a Hewlett-Packard 5890 gas chromatograph. The injection port was kept at 215°C and set for splitless mode for the first 75 s, at the beginning of which the sample was injected, and later set to split mode. We used helium as the carrier gas at a head column pressure of 5.8 psi, and a Chirasil-Val fused silica column (25 m × 0.39 mm × 0.25 mm) from Chrompack. The programme we used was as follows: 50°C (1 min), heat at 40°C min^{-1} to 115°C, remaining at 115°C for 12 min, heating at 3°C min^{-1} to 190°C, remaining at 190°C for 10 min, cooling down to 50°C and remaining at this temperature between runs (80°C if the time between runs is longer, typically overnight). The detector was a FID set at 300°C. Integration of the peak areas was carried out using the PEAK96 integration program from Hewlett-Packard, which runs on a PC computer. 44 shows a typical gas chromatogram of a sample. The sensitivity limits of the method may be fixed

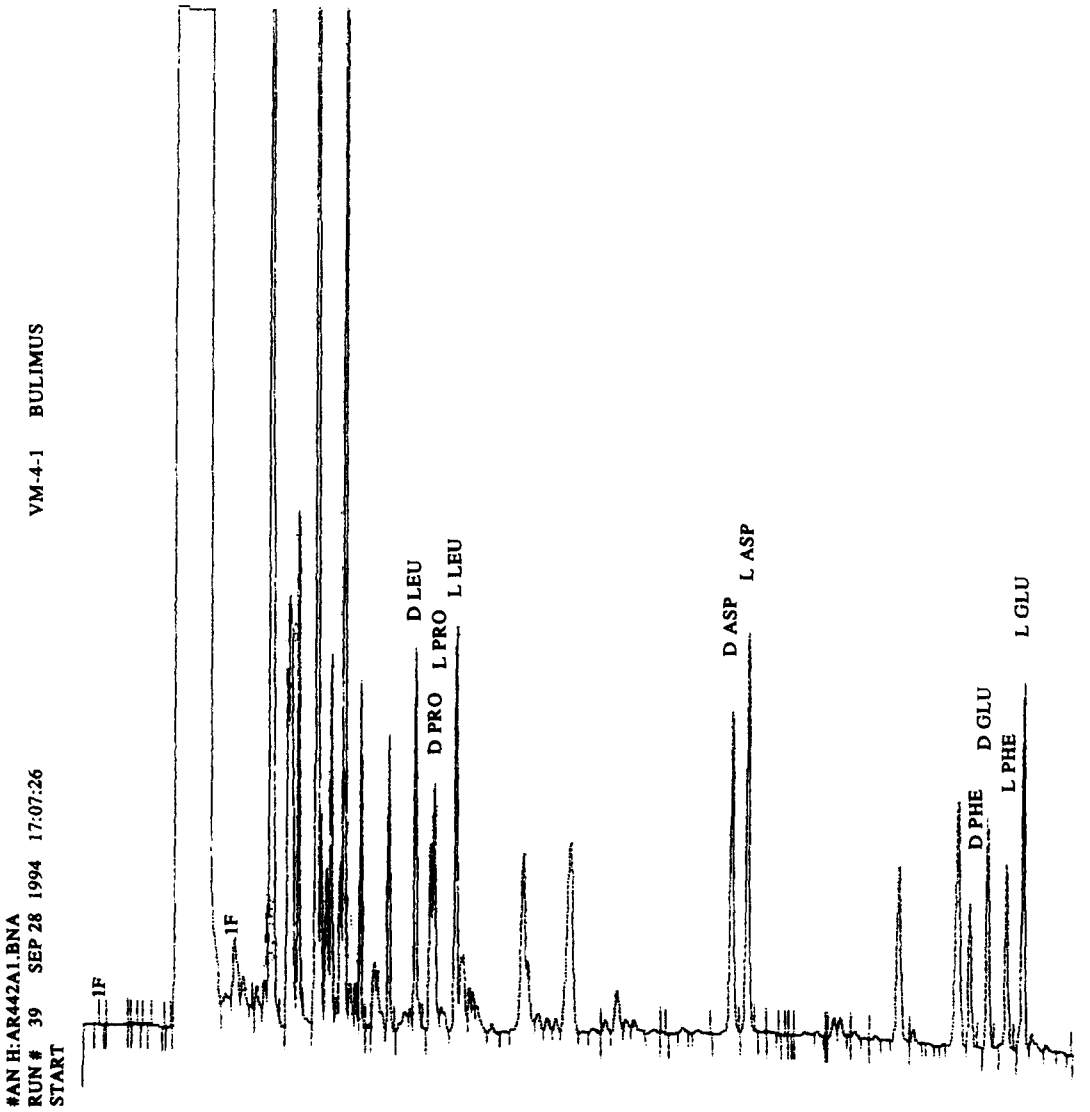

Fig. 4. Typical chromatogram from a *Bulimus* sp. sample from Venta Micena.

Table 1. Comparision of D/L ratios between the interlaboratory results exercise and the results obtained by the amino acid racemization laboratory of E.T.S.I., Minas

Amino acid		D/L [sample (powder)]		
		ILC-A	ILC-B	ILC-C
A/I	ILR	0.212 ± 0.072	0.54 ± 0.0162	1.215 ± 0.030
	LAB	0.180	0.650	1.245
PRO	ILR	0.278 ± 0.068	0.595 ± 0.210	0.81 ± 0.26
	LAB	0.195	0.507	0.786
LEU	ILR	0.196 ± 0.042	0.497 ± 0.098	0.833 ± 0.086
	LAB	0.182	0.444	0.849
ASP	ILR	0.378 ± 0.056	0.705 ± 0.056	0.894 ± 0.158
	LAB	0.373	0.728	0.914
PHE	ILR	0.239 ± 0.040	0.583 ± 0.059	0.873 ± 0.178
	LAB	0.220	0.608	0.885
GLU	ILR	0.203 ± 0.022	0.432 ± 0.034	0.849 ± 0.070
	LAB	0.185	0.426	0.832

ILR = Interlaboratory results (confidence interval was calculated as 2σ).
LAB = laboratory E.T.S.I., Minas.

either in accordance with the racemization induced by the method (1–5%, Table 2, "Today" row) or with the minimum concentration of amino acid detected, which is not a limiting factor (it depends on the age of the samples, since it affects the more recent samples in which the D-amino acid might not be detected). In all the samples analysed to date this problem has been solved by weighing a larger amount of the sample.

Dating method calibration

After setting up the analytical method in our laboratory, we worked to obtain an external validation of the method in order to control any kind of systematic error that might introduce a bias in the results. Three samples from an interlaboratory amino acid racemization exercise (Wehmiller, 1984) were also analysed: ILC-A (*Saxidomus* sp. ca. 50 Ky), ILC-B (*Mercenaria* sp. between 100 and 250 Ky) and ILC-C (*Mercenaria* sp. ca 1.000 Ky). These samples were analysed by 11 laboratories in an exercise of interlaboratory control. The lack of bias and the validity of the process fit provided by our laboratory were confirmed (Table 1).

Samples of molluscs from U/Th method dated Pleistocene fluvial travertine terraces in the Priego area (Torres *et al.*, 1994) were collected, prepared and analysed. The age of the sampled terraces ranged between 6 and 113 Ky. In order to calculate the racemization induced during sample preparation, seven samples of gastropods collected live were also prepared and analysed. With a view to covering older geological records, D/L amino acid ratios from 100 to 250 Ky and ca. 1000 Ky old localities (Wehmiller, 1984) were also used.

Spurious results were observed during interpretation of chromatographic analysis of the samples from Priego: anomalous D/L ratios in samples from the same stratigraphic level (travertine terrace). All the observed deviations appeared as an excess of D-amino acids and seemed to be related to the observed algae–fungi mat covering some of the sampled gastropod shells. Some bacteria have D-amino acids in their cellular walls (Leive and Davis, 1980) and algae and fungi mats are usual bacteria housers. By the time Venta Micena was sampled, we had already modified our recovery process in order to reduce the danger of contamination: the samples were obtained from deeper parts of the strata where there is no influence by the atmosphere and sunlight. In order to avoid the influence of the spurious values, a previous single regression analysis of the D- and L-amino acid pairs in the samples was performed. Table 2 shows the average ratios of racemization of Priego samples.

Finally, prediction models were calculated using a set of 30 U/Th-dated samples from Priego: 21 freshwater gastropods, six terrestrial gastropods and three freshwater pelecipoda, as well as three samples of an interlaboratory comparison exercise (Wehmiller, 1984). The racemization models are based on a first-order reversible reaction (Bada and

Table 2. Average D/L ratios of mollusc samples from U/Th-dated travertine terraces in Priego (Cuenca, Central Spain)

AGE average (U/Th)	A/I	Leu D/L	Asp D/L	Phe D/L	Glu D/L
Today	0.000 ± 0.000	0.008 ± 0.0002	0.051 ± 0.008	0.019 ± 0.011	0.016 ± 0.012
6.000		0.041 ± 0.008	0.215 ± 0.051	0.029 ± 0.044	0.028 ± 0.022
12.500	0.149 ± 0.078	0.071 ± 0.018	0.257 ± 0.019	0.079 ± 0.009	0.070 ± 0.006
20.000		0.133 ± 0.009	0.346 ± 0.040	0.121 ± 0.031	0.105 ± 0.013
105.000	0.237 ± 0.016	0.423 ± 0.026	0.594 ± 0.026	0.479 ± 0.040	0.252 ± 0.031

Protchs, 1973; Schroeder and Bada, 1976; Bada, 1985), the algorithms being based on a time square-root ($\sqrt{t}$) adjustment (Goodfriend, 1987). This would appear to be justified since its use stabilizes the variance of the error that, in the case of time (t) adjustment, becomes progressively greater with growing age samples.

The models obtained are as follows:

$$\text{LEU } \sqrt{t} = (1.17 \pm 0.62) + (11.38 \pm 0.88) \times \ln\{[1 + (D/L)]/[1 - (D/L)]\},$$
correlation coefficient = 0.9774 ($n = 33$)

$$\text{ASP } \sqrt{t} = (-2.17 \pm 1.02) + (10.02 \pm 0.85) \times \ln\{[1 + (D/L)]/[1 - (D/L)]\},$$
correlation coefficient = 0.9740 ($n = 32$)

$$\text{PHE } \sqrt{t} = (0.99 \pm 0.78) + (10.26 \pm 0.74) \times \ln\{[1 + (D/L)]/[1 - (D/L)]\},$$
correlation coefficient = 0.9798 ($n = 34$)

$$\text{GLU } \sqrt{t} = (2.16 \pm 0.65) + (12.44 \pm 0.82) \times \ln\{[1 + (D/L)]/[1 - (D/L)]\},$$
correlation coefficient = 0.9816 ($n = 36$)

The algorithm selected for the isoleucine epimerization model, following the adjustment of different models, was the one commonly proposed by numerous authors (Mitterer, 1975; Goodfriend and Mitterer, 1988; Goodfriend and Meyer, 1991). In this case the best fit was obtained through time (t) adjustment (c.c. 0.9872) rather than time square-root (c.c. 0.9533). The model is as follows:

$$\text{A/I } t = (-34.99 \pm 25.8) + (267.14 \pm 20.32) \times \ln\{0.565/[0.565 - (A/I)/(1 + A/I)]\},$$
correlation coefficient = 0.9872 ($n = 20$)

Although the final equilibrium state is affected only by temperature, for the Ile/Alo epimerization reaction it demonstrated the existence of a relationship in Foraminifera between racemization percentages, age and the current mean annual temperature (CMAT) (Wehmiller, 1984). In our study, we have determined the CMAT for the areas of Priego (Torres *et al.*, 1994) and Redueña (Llamas *et al.*, 1995), which are climatologically similar to the zone under study, a CMAT of 11–14°C having been found. Thus, it has been considered that the same models would be applicable. As regards the type of fossils under study, the initial results pointed to the existence of differences between amino acid kinetics depending on the genera of mollusca analysed. These differences were more marked in the oldest samples analysed. In 10 samples of ancient freshwater gastropoda (*Planorbis* sp. and *Radix* sp.) from Priego (Torres *et al.*, 1994) this effect was very marked for glutamic acid but negligible for leucine. A poor correlation between the racemization ratios of different amino acids might be explained in terms of early diagenesis (Goodfriend, 1991), e.g. the loss of the most easily hydrolyzable amino acid from the terminal protein chains, aspartic acid, might be reflected in lower D/L ratios, this apparently being uncorrelated with the higher D/L ratios of the less easily hydrolyzable amino acids, such as isoleucine.

Ten individual samples from the Priego area were dated according to the D/L leucine ratios, and an average value of 733 ± 140 ky was obtained. For the Priego area a current mean annual temperature (CMAT) of between 11 and 14°C was obtained. These values were coherent with those corresponding to this geographical location, and the CMAT was used according to the Wehmiller (1993) criteria. From our point of view, temperature might affect only the final Alo/Ile equilibrium stage. According to these results, other algorithm families were adjusted using only *Planorbys* sp. and *Radix* sp. sample results, the adjusted models being as follows:

$$\text{LEU } \sqrt{t} = (0.94 \pm 0.63) + (11.81 \pm 0.70) \times \ln\{[1 + (D/L)]/[1 - (D/L)]\},$$
correlation coefficient = 0.9870 ($n = 32$)

$$\text{ASP } \sqrt{t} = (-3.34 \pm 2.31) + (12.38 \pm 2.24) \times \ln\{[1 + (D/L)]/[1 - (D/L)]\},$$
correlation coefficient = 0.9050 ($n = 29$)

$$\text{PHE } \sqrt{t} = (0.48 \pm 2.88) + (15.64 \pm 3.71) \times \ln\{[1 + (D/L)]/[1 - (D/L)]\},$$
correlation coefficient = 0.8304 ($n = 34$)

$$\text{GLU } \sqrt{t} = (0.33 \pm 1.44) + (21.93 \pm 2.48) \times \ln\{[1 + (D/L)]/[1 - (D/L)]\},$$
correlation coefficient = 0.9476 ($n = 37$)

In this case the best model found for Alo/Ile epimerization was the square-root of time ($\sqrt{t}$) adjusted (c.c. 0.9495). Time (t) adjustment had a lower correlation cofficient value (c.c. 0.9359).

$$\text{A/I } \sqrt{t} = (-0.02 \pm 1.42) + (21.85 \pm 2.31) \times \ln\{0.565/[0.565 - (A/I)/(1 + A/I)]\},$$
correlation coefficient = 0.9495 ($n = 21$)

RESULTS AND DISCUSSION

Venta Micena

At Venta Micena, only 21 analytical results were obtained from the 23 original samples, two being lost during the sample preparation process. The

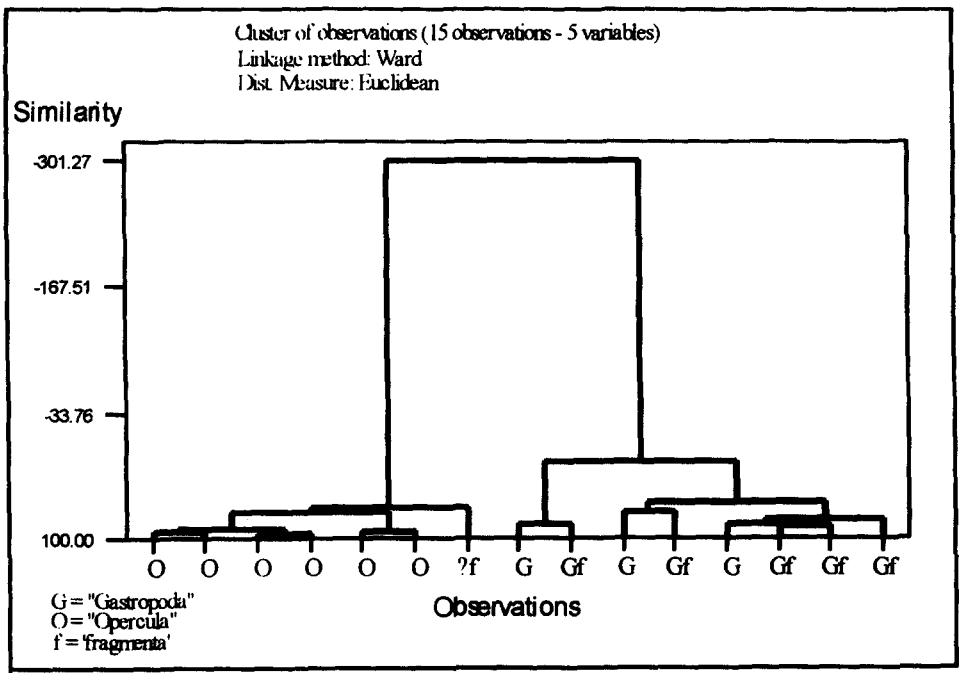

Fig. 5. Similarity cluster of amino acid racemization ratios (Ile/Alo isoleucine, leucine, aspartic acid, glutamic acid) of Venta Micena samples.

remaining samples could be classed into two groups: *Opercula* and *Gastropoda*. The former were 2–3 mm sized, subcircular-ellipsoidal shaped dishes of aragonite, probably of *Bithynia* sp. Three different genera were grouped into *Gastropoda*.

First, a cluster analysis was performed with GC analysis results, three different groups being identified. One of these groups, which differed widely from the other two, included four samples with very low racemization rates, this disagreeing with

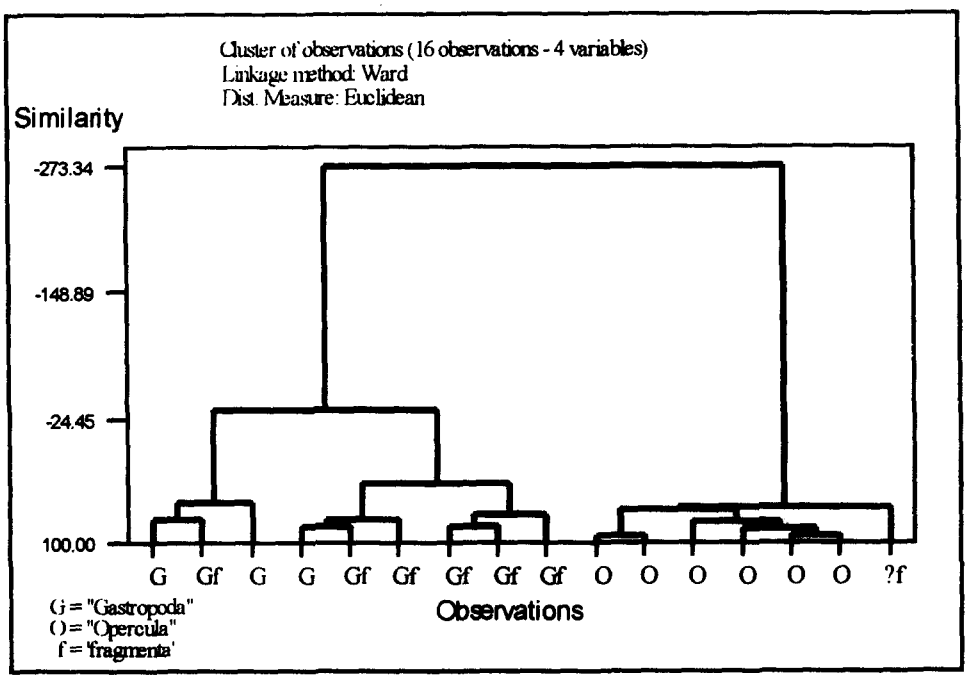

Fig. 6. Similarity cluster of amino acid racemization ratios (Ile/Alo isoleucine, leucine, aspartic acid, phenilalanine, glutamic acid) of Venta Micena samples.

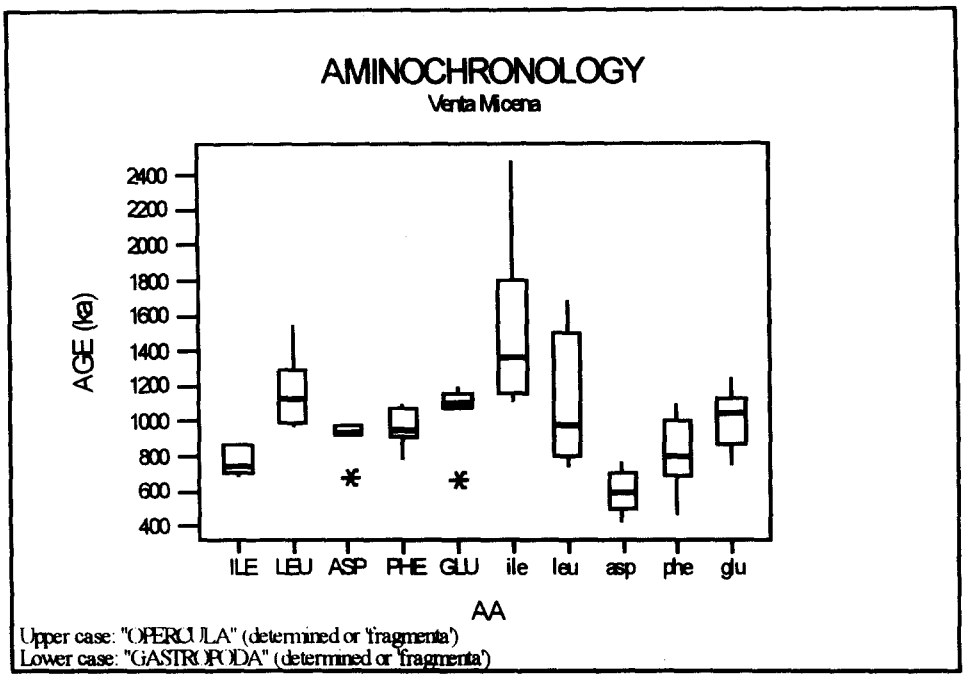

Fig. 7. Box and whisker plot of calculated ages from different amino acids and materials from Venta Micena.

the other two. These results were interpreted as being due to the influence of contamination, and were discarded. A second cluster analysis of the 17 remaining samples revealed the existence of two groups: *Opercula*, along with an unidentified *fragmenta*, and *Gastropoda* (two *Radix* sp., one *Bulimus* sp. and six unidentified gastropod *fragmenta*). In the second group a *fragmenta* sample presented very low racemization ratios, with the exception only of Alo/Ile. This result has been interpreted as a diagenetic effect and was also discarded prior to performance of the final cluster analysis (Fig. 5). All the analyses were carried out on the basis of four amino acids (Alo/Ile, leucine, aspartic acid and glutamic acid) since some results for phenylalanine were missing. When the clustering was accomplished using complete results, phenylalanine included, the grouping obtained was the same (Fig. 6), thus reinforcing the validity of the previous cluster analysis.

Final age calculation was accomplished according to the first set of models for seven samples grouped into *Opercula*, and according to the second set of models for nine samples included in the *Gastropoda* group (Fig. 7 and Table 3). The unidentified *fragmenta* was included in the *Opercula* group, this giving rise to a slight difference with respect to previously published data (Torres *et al.*, 1995).

Taking into account the fact that the results obtained are independent measurements of the same parameter over a time interval, it is possible to calculate a global dating for each group (Table 3); it may be observed that age calculations for the *fragmenta* group show a higher scattering than those for the *Opercula* group. Prior to computing a "general average dating", the former global results from (Table 3) were analysed for each sample group, *Opercula* and *Gastropoda*. The test of the homogeneity of variances for the two groups shows that both are different, according to the

Table 3. D/L average values and age calculations of Venta Micena samples

	A/I	LEU	ASP	PHE	GLU	GLOBAL
			Opercula			
D/L	1.160 ± 0.031	0.893 ± 0.017	0.922 ± 0.012	0.896 ± 0.013	0.835 ± 0.033	
Age (Ky)	778 ± 66	1174 ± 149	947 ± 18	909 ± 78	1062 ± 138	976 ± 65
			Gastropoda			
D/L	0.873 ± 0.055	0.881 ± 0.043	0.803 ± 0.023	0.706 ± 0.043	0.613 ± 0.025	
Age (Ky)	1511 ± 318	1111 ± 258	591 ± 77	809 ± 144	1012 ± 107	1009 ± 130

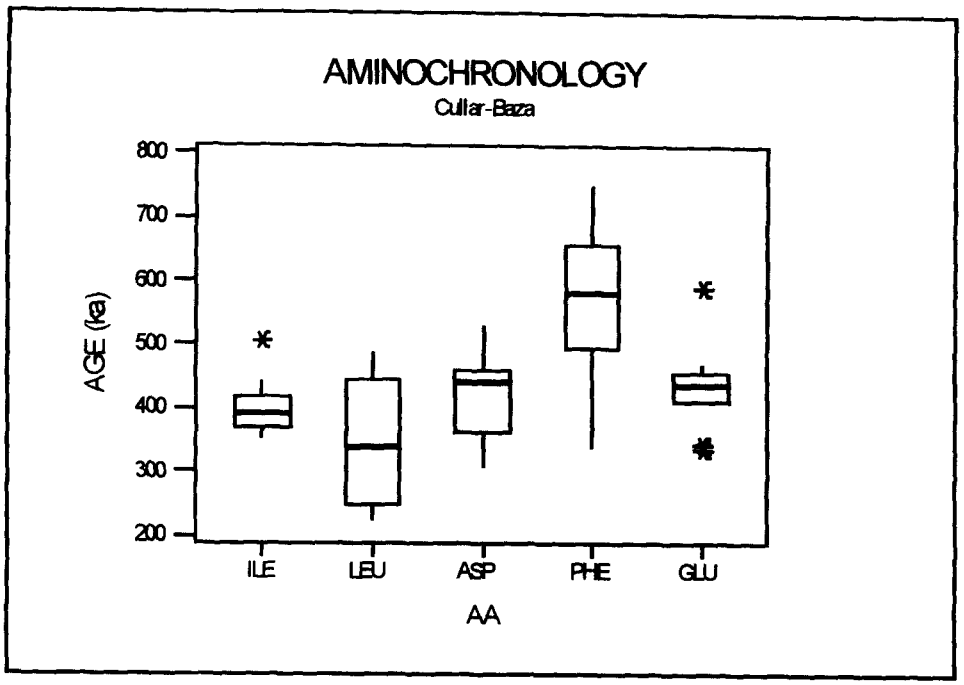

Fig. 8. Box and whisker plot of calculated ages from different amino acids and materials from Cúllar Baza.

Levene tests (p value = 0.002). The test for the equality of the means, taking into account the non-homogeneity of variance, demonstrates that the non-equability hypothesis cannot be rejected (p = 0.65).

To estimate the global mean from the means of the two sample groups, *Opercula* (1001 Ky) and *Gastropoda* (933 Ky), a weighted average has been calculated using the inverse of variances (1043 and 4212 Ky^2) as weighting factors. The result obtained was 983 Ky, with a variance of 836 Ky^2 (983 $\pm$ 58 Ky for the 95% confidence interval).

Cúllar-Baza

Fifteen *Gastropoda* samples were analysed (14 *Helix* sp. and one *Planorbis* sp.); one of them was lost during the sample preparation process. The cluster analysis of the GC results and D/L ratios showed the existence of an isolated group, and an anomalous individual with lower D/L ratios for all the amino acid pairs identified, this being rejected. Final dating was calculated from the 13 remaining sample results. According to our own experience (Torres *et al.*, 1995), the amino acid racemization kinetics of the *Helix* genus could assimilated to the first model set; calculations performed using the inadequate model set produced incoherent results, the only exception being leucine. Dating calculations were performed from each amino acid D/L pair, see Fig. 8, and with all of them grouped. An age of 441 $\pm$ 27 Ky was obtained (Table 4).

CONCLUSIONS

The AARD method worked accurately in the Guadix-Baza basin: the data obtained are located on the chronostratigraphical levels assumed (using classical palaeontological methods) or slightly younger. An Upper Villafranchian geological age (Middle Biharian or 31 oxygen isotopic stage) may clearly be assumed for the Venta Micena palaeontological site; likewise, the Lower Villafranchian situation of the Venta Micena fauna remains may be entirely rejected. The dating of the Cúllar-Baza site,

Table 4. D/L average values and age calculations of Cúllar-Baza samples

	A/I	LEU	ASP	PHE	GLU	GLOBAL
Helix D/L	0.828 $\pm$ 0.030	0.610 $\pm$ 0.054	0.809 $\pm$ 0.016	0.799 $\pm$ 0.027	0.633 $\pm$ 0.019	
Age (Ky)	399 $\pm$ 23	351 $\pm$ 64	421 $\pm$ 36	569 $\pm$ 66	435 $\pm$ 34	441 $\pm$ 21

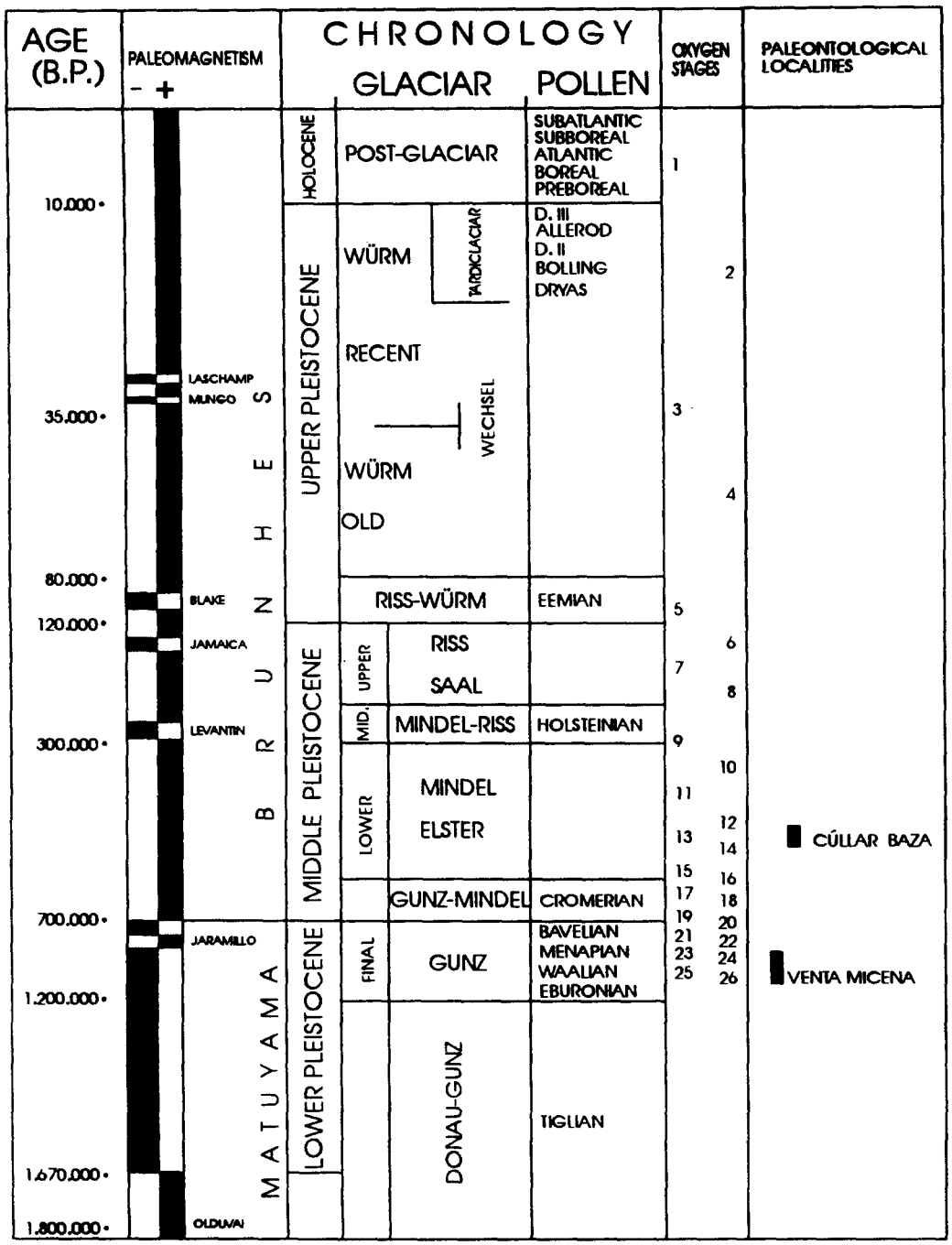

Fig. 9. Chronostratigraphical situation of Venta Micena and Cúllar Baza localities.

Middle Pleistocene (Upper Biharian, Mindel or 12–13 oxygen isotopic stages), was also coherent (Sese, 1993), and certifies the validity of the Venta Micena dating fit. The results are summarized in (Fig. 9).

Acknowledgements—This study has beeen partially supported by UPM-ENRESA contract no. 0701041 "Datación de Formaciones Cuaternarias a partir de aminoácidos" (Dating of Quaternary Formations on the basis of amino acids). Cúllar-Baza site dating was obtained from the Project "Mapa Geológico Nacional de España 1:50000" of the Instituto Tecnológico Geominero de España.

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